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## **Evolutionary processes from the perspective of flowering time diversity**

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## 40 **Summary**

41 Although it is well appreciated that genetic studies of flowering time regulation have led to  
42 fundamental advances in the fields of molecular and developmental biology, the ways in which  
43 genetic studies of flowering time diversity have enriched the field of evolutionary biology have  
44 received less attention despite often being equally profound. Because flowering time is a complex,  
45 environmentally responsive trait that has critical impacts on plant fitness, crop yield, and reproductive  
46 isolation, research into the genetic architecture and molecular basis of its evolution continues to yield  
47 novel insights into our understanding of domestication, adaptation, and speciation. For instance,  
48 recent studies of flowering time variation have reconstructed how, when, and where polygenic  
49 evolution of phenotypic plasticity proceeded from standing variation and *de novo* mutations; shown  
50 how antagonistic pleiotropy and temporally varying selection maintain polymorphisms in natural  
51 populations; and provided important case studies of how assortative mating can evolve and facilitate  
52 speciation with gene flow. In addition, functional studies have built detailed regulatory networks for  
53 this trait in diverse taxa, leading to new knowledge about how and why developmental pathways are  
54 rewired and elaborated through evolutionary time.

55

56 **Keywords:** evolution, flowering time, adaptation, domestication, speciation, evo-devo, phenotypic  
57 plasticity, phenology

58

59

## 60 **I. Introduction**

61 Timing the initiation of reproductive development appropriately in the context of seasonally changing  
62 conditions is critical for fitness. In angiosperms, if flowering occurs too early, floral tissues may be  
63 damaged by late frosts, pollinators and other flowering conspecifics may not yet be abundant enough  
64 to ensure all ovules are fertilized, and plant size may constrain total flower production. If flowering  
65 occurs too late, a plant may encounter conditions unfavorable for seed maturation or dispersal, fail to  
66 set seed before dying in season-ending frosts or droughts, or leave offspring in poor growth  
67 environments.

68 As a consequence of these and other time-dependent factors that influence survival,  
69 fecundity, and gene flow, plants have evolved mechanisms to regulate their seasonal reproductive  
70 phenology—when flowering begins and ends—through internal timekeepers and environmental  
71 signals. Developmental plasticity of flowering time to environmental signals is particularly important in

temperate environments, as these mechanisms allow plants to sense changing signals highly predictive of growing season timing and actively adjust flowering accordingly. For instance, many plants require prolonged exposure to cold, or vernalization, for floral induction as this indicates the passage of winter and avoids mortality that would result from prematurely flowering in the fall (Bouché *et al.*, 2017). In many annual plants, a permanent memory of winter is established through stable epigenetic silencing of factors that repress flowering, but in perennials, this silencing is not stable, allowing new vegetative growth that will flower in future years (Hyun *et al.*, 2019).

Obligate or facultative flowering responses to photoperiod, a reliable indicator of calendar date compared to other environmental inputs, are also common adaptations to ensure the appropriate seasonal timing of reproduction. Photoperiod measurement is achieved by the circadian gating of light- or dark-inducible signaling mechanisms, such that only when days are above or below the required length is the flowering regulatory signal transmitted (Song *et al.*, 2015). Pathways involving epigenetic regulation have also been discovered that track plant age to prevent seedlings from flowering prematurely (Hyun *et al.*, 2017). These pathways converge to regulate several floral inducers expressed in the shoot apical meristem and also a mobile hormonal signal known as florigen, which is encoded by homologs of the FLOWERING LOCUS T (FT) protein and moves from the leaf to the shoot apex to induce flowering (Andrés & Coupland, 2012).

Because the start and duration of the growing season vary across the landscape and between wild and agronomic environments, flowering time and its regulation by environmental cues are frequent targets of and contributors to evolutionary processes operating within and between species. Due to its agronomic importance, regulation of flowering time has received intensive study in model organisms and diverse crop species (Andrés & Coupland, 2012; Blackman, 2017). The highly detailed knowledge of developmental mechanisms produced by this immense body of work has facilitated abundant research into the genetic underpinnings of the processes of domestication, adaptation, and speciation and also allowed for comparisons at macroevolutionary scales that have informed our understanding of how developmental networks evolve. Here, we review how genetic studies of flowering time variation have advanced our understanding of these key evolutionary processes, with particular emphasis on recent work and on synthesizing findings across study systems. We conclude by highlighting areas where genetic studies of flowering time variation are poised to make major contributions to evolutionary biology in the future.

## II. Domestication

104 The evolutionary transformation of wild plants into crops is a multi-stage process (Kantar *et al.*, 2017;  
105 Gaut *et al.*, 2018). During initial domestication, early farmers began managed cultivation of a species  
106 and consciously or unconsciously selected on harvest and yield traits. Next, cultivation spread from  
107 the center(s) of origin, and during this dispersion stage, adaptation to local environmental conditions  
108 was often essential. Finally, during modern improvement, breeders have further altered crops  
109 through artificial selection on natural variants or induced mutants. Flowering time and its  
110 environmental regulation have often been targets of selection during these processes, as altering  
111 these traits and their underlying pathways can impact critical components of yield including seasonal  
112 phenology, plant architecture, and developmental synchrony at harvest.

113 Here, we primarily focus on the evolution of flowering time during initial domestication and  
114 dispersion, as these stages share greatest similarity to evolutionary processes operating in natural  
115 populations (Purugganan & Fuller, 2009). We first take a trait-centric perspective, drawing on  
116 literature from diverse crops. In doing so, we aim to illustrate how flowering time evolves as a  
117 multifaceted phenotype comprised of genetically dissociable regulatory modules that integrate  
118 information from multiple seasonal cues and developmental timekeepers (Blackman, 2017). Then,  
119 we specifically describe how the evolution of photoperiodic flowering during maize dispersion  
120 provides a compelling case study of polygenic adaptation, as knowledge of causal variants reveals  
121 how evolution of this trait accumulated over space and time.

### 122 123 *Evolving long-day annuals into crops by reducing photoperiod and vernalization responses*

124 Many herbaceous annual species germinate in the fall and overwinter before flowering in the spring.  
125 In these taxa, vernalization is often required to relieve the repression of flowering established early in  
126 development, and long-day photoperiods activate floral inducers and/or relieve other repressors of  
127 those inducers. However, spring plantings are often favored in agriculture, and the pressure to flower  
128 quickly in the spring may be reduced or absent in an agronomic context depending on environmental  
129 conditions. Thus, substitutions that attenuate these inductive responses have been favored in some  
130 crops since delayed flowering can improve resource utilization and yield with extended growing  
131 seasons (Table 1).

132 For instance, many spring-sown varieties of barley (*Hordeum vulgare*) and einkorn wheat  
133 (diploid *Triticum monococcum*) differ from winter-sown varieties by regulatory mutations, missense  
134 substitutions, or even full deletions of *VRN2* (*VRN-H2*), a pseudo-response regulator (PRR; Yan *et al.*, 2004). Functional *VRN2* represses floral inducer expression until it is down-regulated by *VRN1*

(*VRN-H1*), a MADS-box transcription factor expressed in response prolonged cold. Therefore, spring varieties of these grains can flower without vernalization. Other spring einkorn wheat varieties carry deletion variants in the *VRN1* promoter (Yan *et al.*, 2003, 2004). Spring-sown barley varieties also segregate for a missense variant in another PRR, *Ppd-H1*, which delays flowering under long days by preventing induction of the *FT* homolog *HvFT1*. The frequency of the missense allele varies clinally such that non-responsive genotypes are more common at more northern latitudes, where mild summer conditions make for longer growing seasons (Turner *et al.*, 2005; Jones *et al.*, 2008).

In contrast, in winter varieties of wheat (polyploid *Triticum aestivum* ssp. *aestivum*), photoperiod response is fine-tuned among populations by copy number variants (CNVs) and allelic combinations of homeologs of *Ppd-H1* (Bentley *et al.*, 2013; Würschum *et al.*, 2015, 2018). Adding copies accelerates flowering under non-inductive short days, allowing southern European populations to initiate reproduction and grain filling earlier in the calendar year to escape end-of-season summer heat and drought. Quantitative variation in long-day response mediated by earlier flowering under non-inductive short days is also observed across cultivars of two domesticated legumes, pea (*Pisum sativum*) and lentil (*Lens culinaris*). In both cases, variants that produce frameshifts in orthologs of *EARLY FLOWERING 3* (*ELF3*), a component of the circadian clock, are responsible (Weller *et al.*, 2012)

#### Adapting short-day crops to shorter growing seasons by altering photoperiod response

Environments with short growing seasons or with long end-of-season photoperiods pose challenges for crops domesticated from short-day wild ancestors, as flowering too late risks mortality from early frosts prior to maturity. Therefore, for many such species, domestication or dispersal to higher latitudes or altitudes has selected for variants that abolish or reduce the strength of the short-day flowering response (Table 1). For instance, two recent elegant studies in tomato (*Solanum lycopersicum*) attribute the shift from a strong short-day response to early, nearly day-neutral flowering to allelic variation in *SELF PRUNING 5G* (*SPG5*), a paralog of the tomato *FT* ortholog *SINGLE FLOWER TRUSS* (*SFT*) (Soyk *et al.*, 2017; Zhang *et al.*, 2018). *SP5G* has evolved to function as a repressor of flowering in long days, but a 52-bp deletion in a 3' UTR enhancer region, which causes improper transcript termination rather than reduced transcript initiation, shows evidence of selection during domestication (Zhang *et al.*, 2018).

Recent evidence suggests that allelic variation in a *FT* homolog may also be responsible for the evolution of earlier flowering under non-inductive long days in day-neutral temperate cultivars of

sorghum (*Sorghum bicolor*; Cuevas *et al.*, 2016). Another example of mechanistic convergence is observed in temperate-adapted cultivars of short-day rice (*Oryza sativa*), where induction of early flowering under non-inductive photoperiod conditions occurs in a manner parallel to the barley and wheat examples above, i.e., through missense or null alleles in orthologs of the PRRs *VRN2* and *Ppd-H1* (Xue 2008, Yan 2013, Koo 2013). Finally, convergent evolution at the genetic level is also observed in legumes. In soybean (*Glycine max*) and independently in both domestications of common bean (*Phaseolus vulgaris*), loss-of function alleles in homologs of the light receptor *PHYTOCHROME A* have been implicated in the evolution of photoperiod insensitivity (Xu *et al.*, 2013; Jiang *et al.*, 2014; Weller *et al.*, 2019).

177

#### 178 *Polygenic evolution during range expansion*

179 Since its initial domestication ~9000 years ago from short-day teosinte (*Zea mays ssp. parviglumis*),  
180 maize (*Z. mays* L.) spread from the tropical lowland Balsas River basin into temperate and higher  
181 latitude areas of North America where it faced many of the same climate-associated challenges as  
182 the crops discussed above (Swarts *et al.*, 2017). We give special focus to maize here because  
183 recent functional, quantitative genetic, population genomic, and archaeological DNA studies have  
184 together made exceptional progress in unraveling how this unfolded. Maize landraces vary from 35-  
185 120 days to flower. This variation is highly polygenic, involving thousands small effect polymorphisms  
186 (most alter flowering time by <1 day) that often also show population genomic signatures of  
187 latitudinal or altitudinal adaptation (Buckler *et al.*, 2009; Romero Navarro *et al.*, 2017). Many of these  
188 polymorphisms are in regions of low recombination, and recent work has determined that time to  
189 flowering and genome size are positively correlated, a pattern driven predominantly by variation in  
190 the number of large heterochromatin knobs (Bilinski *et al.*, 2018) and that highlights how  
191 chromosome-scale differences may influence adaptation (Fig. 1a). Even discounting SNPs whose  
192 association with flowering variation solely reflects linkage disequilibrium with these larger structural  
193 features, there are likely still hundreds of adaptive flowering time variants in maize.

194 Five genes contributed specifically to the reduction of short-day response as maize cultivation  
195 spread northward, and most causal variants are known. For instance, upstream of *ZNC8*, the maize  
196 *FT* homolog that promotes flowering under short days, a nucleotide substitution and a small deletion  
197 are each associated with higher gene expression and earlier flowering under long days (Fig. 2a; Guo  
198 *et al.*, 2018). The former is nearly fixed in maize landraces throughout the Americas and segregates  
199 within teosinte subspecies, suggesting an early sweep from standing variation. The latter is only



found on haplotypes with the early allele of the former, is at higher frequencies in northern landraces, and segregates only within *Z. mays ssp. mexicana*, consistent with adaptive introgression from this highland teosinte subspecies facilitating northward dispersion. Two genes—*ZmCCT9* and *ZmCCT10*—are PRRs homologous to *VRN2*, and loss-of-function alleles caused by transposable element (TE) insertions in their promoters are more frequent at higher latitudes. These alleles cannot repress their downstream target *ZCN8* (Hung *et al.*, 2012; Yang *et al.*, 2013; Huang *et al.*, 2018b). Likewise, a TE insertion at higher frequency in Northern populations disrupts the function of *Vgt1* (or *ZmRap2.7*), an AP2/ERF transcription factor that represses *ZCN8* expression in long days (Ducrocq *et al.*, 2008). All these insertions appear to have occurred *de novo* post-domestication; a molecular evolutionary analysis of the terminal repeats of the *ZmCCT9* and *ZmCCT10* TE insertions dated their origins to ~4645 and ~7269 years before present (ybp), respectively (Huang *et al.*, 2018b). The MADS-box transcription factor *ZmMADS69*, which promotes flowering by repressing *Vgt1*, also shows a signature of a selective sweep associated with domestication, but the causal variant(s) is unknown (Liang *et al.*, 2019).

This exciting composite, interdisciplinary body of research demonstrates how adaptive changes accreted throughout a regulatory network as selection drove the evolution of day-neutral flowering to short-day flowering during as maize cultivation spread northward. (Fig. 2b), yielding ample insight into evolutionary process and illustrating how evolutionary change during the dispersion stage of domestication could be highly similar to climate adaptation in wild populations. The common involvement of *de novo* TE insertions suggests that these mobile elements are potent sources of new adaptive loss-of-function variants. Furthermore, the combined involvement of standing variants and *de novo* post-domestication alleles in building multi-locus genotypes with temperate-adapted phenologies is consistent with the delayed expansion of maize agriculture out of the southwest United States for a couple millennia (Swarts *et al.*, 2017). Notably, a sample of archaeological maize cobs from that period (~1900 ybp) is fixed for all the early flowering variants except the *Vgt1* TE. However, counter to expectation, two Southern Mexican genomes dating to ~5000 ybp carry both *ZmCCT* TEs but have the late flowering *ZCN8* alleles (Guo *et al.*, 2018). Thus, a richer archaeological time series may reveal additional aspects of domestication and dispersion that other processes have obscured from modern genomes over time.

### III. Adaptation

231 Natural selection improves the fit of organisms to their environments. However, the environment  
232 varies over space and time, and consequently, individual populations within species may diverge  
233 phenotypically as they adapt to their local habitats and may maintain trait variation across  
234 generations. Each of these adaptive processes may involve changes at few or many loci; be biased  
235 toward or away from particular genes or types of substitutions; and could result from several  
236 evolutionary mechanisms. Consequently, to know what underlying processes can and have  
237 occurred, it is essential to dissect the genetic architecture and/or molecular basis of adaptive trait  
238 variation and examine genotypic effects in natural environments.

239         Flowering time is a highly tractable model trait for examining these aspects of the adaptation  
240 process. Common garden studies have revealed that genetic clines in flowering time with elevation,  
241 latitude, or other climate parameters are common (e.g., Kollmann & Bañuelos, 2004; Stinchcombe *et*  
242 *al.*, 2004; Kawakami *et al.*, 2011; Van Dijk & Hautekèete, 2014), and shifts to rapid cycling life  
243 histories frequently accompany adaptation to environments with seasons abbreviated by terminal  
244 drought or seasons long enough to sustain multiple annual life history cycles (e.g., Donohue, 2002;  
245 Baduel *et al.*, 2016; Ferris *et al.*, 2017). The responsiveness of flowering to vernalization,  
246 photoperiod, and ambient temperature cues also varies in ways both similar and distinct to the  
247 patterns observed among geographically widespread crop species (Lempe *et al.*, 2005; Blackman *et*  
248 *al.*, 2011; Anderson *et al.*, 2011; Ream *et al.*, 2014; Kooyers *et al.*, 2015). Early progress in  
249 examining the genetics of flowering time diversity was dominated by work in the model plant  
250 *Arabidopsis thaliana* due to its experimental advantages. However, the development of new  
251 population genomic methods, the expansion of genomic resources, and the extension of functional  
252 tools to other species in the Brassicaceae, wild relatives of crop species, and several classic  
253 evolutionary systems now allow for genetic dissections approaching the scale of individual genes in  
254 these species too. Here, we consider how these studies and some additional relevant findings from  
255 work on crop landrace diversity inform our understanding of adaptive processes operating within and  
256 among populations.

## 257 258 *The genetic architecture of flowering time adaptation*

259 Many investigations have sought to characterize the number and effect sizes of loci that contribute to  
260 adaptive variation in flowering time segregating within single populations or among populations of a  
261 species. These studies contribute to ongoing dialogues about whether adaptation proceeds through  
262 allele frequency changes at many loci of small effect or few loci of major effect; whether and why we

263 see the same or different genes involved in convergent phenotypic evolution; and, thus together, how  
264 to model quantitative trait variation and how quick or how difficult adaptive evolution may be.  
265 Although an ever-growing number of individual studies have explored these questions in diverse  
266 species, a truly robust picture is as yet unavailable for most wild species for several methodological  
267 and biological reasons (Box 1). Nonetheless, when we look at some of the most comprehensive  
268 studies to date, different templates for the genetic architecture of flowering time adaptation have  
269 emerged.

270 For instance, when the genetics of flowering time variation was mapped in *A. thaliana* across  
271 17 F<sub>2</sub> populations derived from 18 unique parent accessions and several additional recombinant  
272 inbred line (RIL) populations, the QTLs identified mostly map to allelic series at five major loci—  
273 including *FRIGIDA* (*FRI*), *FLOWERING LOCUS C* (*FLC*) (and/or nearby *CONSTANS* (*CO*)), and two  
274 paralogs of *FLC* (*FLOWERING LOCUS M* and *MADS AFFECTING FLOWERING 2*), and  
275 *ERECTA*—plus rarer alleles at several other loci including *FT* (Salomé *et al.*, 2011). *FRI* is a positive  
276 regulator of *FLC*, which represses flowering until silenced by vernalization. *CO* is a positive regulator  
277 of *FT* in long days, and the two *FLC* paralogs are involved in the response of flowering time to  
278 ambient temperature. These results suggest that although the flowering time regulatory network is  
279 large and mutants in >300 genes affect flowering (Bouché *et al.*, 2016), adaptation may proceed  
280 primarily through large effect substitutions from a pool of allele diversity harbored at a few predictable  
281 “evolutionary hotspots”, a pattern that may emerge because advantageous alleles with no or few  
282 negative pleiotropic effects arise more frequently at certain loci (Stern, 2013). Consistent with these  
283 patterns, seven additional *FLC* alleles that have sustained independent TE insertions are associated  
284 with reduced gene expression and earlier flowering (Lempe *et al.*, 2005; Quadrana *et al.*, 2016).  
285 Furthermore, natural variants that eliminate, attenuate, or otherwise alter the functions of *FRI*, *FLC*,  
286 and *CO* homologs have been implicated in the adaptive evolution of seasonal flowering in three other  
287 species (Yang *et al.*, 2018; Baduel *et al.*, 2018; Lee *et al.*, 2018) and between species (Kiefer *et al.*,  
288 2017) in another genus in the Brassicaceae.

289 Other studies have called into question whether this major effect “hotspot” architecture is  
290 generalizable, however. For example, although initial association mapping by the 1001 Genomes  
291 Consortium detected just five flowering-time associated loci largely similar to those discussed above  
292 (Alonso-Blanco *et al.*, 2016), more in-depth analyses informed by eQTL analysis of the same set of  
293 accessions have detected ~40 additional loci harboring variants that influence flowering time in  
294 *A. thaliana* (Zan & Carlborg, 2019). Moreover, another survey performed on the same dataset

yielded >80 genes with drought-associated, loss-of-function common variants that may alter flowering time (Monroe *et al.*, 2018), although more recent findings raise cautions about validating the pooled flowering time effects of large candidate genes sets with T-DNA insertion lines (Chong & Stinchcombe, 2019). As noted above, adaptive differentiation in flowering of maize landraces along altitude or latitude gradients may involve hundreds of genes (Romero Navarro *et al.*, 2017). In addition, bulked segregant analyses comparing allele frequencies of early and late flowering plants in three populations of the common monkeyflower, *Mimulus guttatus*, over multiple years detected tens to hundreds of single nucleotide polymorphisms (SNPs) or structural variants that contribute to adaptive variation within and between populations (Monnahan & Kelly, 2017; Troth *et al.*, 2018). However, many SNPs only affected flowering time in one of the populations and/or only in one growing season, a pattern reminiscent of several studies that have found flowering time QTL or variants mapped in growth chamber or greenhouse studies often do not affect flowering or fitness as anticipated in the field (Weinig *et al.*, 2002; Brachi *et al.*, 2010; Anderson *et al.*, 2011; Liu *et al.*, 2014). These results argue for an alternative model of flowering time adaptation where a myriad of loci throughout the various environmentally sensitive pathways of the flowering time regulatory network can harbor adaptive genetic variation, particularly when their gene-by-environment interactions are taken into account.

The disparate models--allelic series at few hotspots vs. highly polygenic--may emerge in part due to methodological differences (e.g., many fewer parental genotypes sampled by QTL mapping vs. GWAS or population genomics) or biological differences (e.g., highly selfing vs. highly outcrossing mating systems). Regardless, this important dichotomy signals that more expansive and comparable investigations of the genetics of adaptation in wild systems are needed. For instance, if adaptive evolution of flowering time is constrained to occur through repeated major effect substitutions at few loci, then adaptation to climate change may be constrained by a limited range of adaptive variants segregating in natural populations or involve long waiting times for the appearance of new advantageous variants. In contrast, if many genes can contribute ecologically equivalent allelic variation to flowering time adaptation and if loss-of-function mutations are often beneficial and occur frequently (e.g., following TE activation by environmental stress), the prospects for adaptation to climate change seem brighter.

*Pleiotropy and fluctuating or spatially varying selection*

326 Substantial inquiry has focused on balancing selection, the adaptive force that maintains  
327 polymorphism within a single population. Classic case studies have shown how heterozygote  
328 advantage and negative frequency dependent selection can sustain balanced polymorphisms (Box  
329 2). Temporally varying selection, where the alleles and trait values favored in some generations are  
330 disfavored in other generations, is another important and potentially more pervasive evolutionary  
331 explanation. Because it is theoretically possible for an allele or multi-locus genotype with high  
332 geometric mean fitness across many generations to fix (thus eliminating any polymorphism) under  
333 fluctuating selective regimes however, antagonistic pleiotropy is often invoked as a critical  
334 requirement for maintaining balanced polymorphisms. In other words, alleles that confer high relative  
335 fitness in some years may suffer a fitness trade-off in other years either due to their direct effects on  
336 the trait that makes them sometimes favorable or indirectly through impacts on other traits.

337 Recent multi-year field studies of flowering time variation in *M. guttatus* have now affirmed  
338 that these conditions are indeed met in wild populations. For instance, one GWAS on a diversity  
339 panel derived from a single population first identified 24 pleiotropic SNPs associated with both  
340 delayed flowering and increased plant size in the greenhouse (Troth *et al.*, 2018). Then, by tracking  
341 them over three field seasons, the investigators found this set of “large and slow” alleles was  
342 maladaptive in shorter, drier seasons, when an early terminal drought leads to mortality before  
343 flowering or seed set, but favored in longer, wetter seasons because plants with delayed flowering  
344 grew larger and produced more, larger flowers, giving them higher fecundity.

345 Likewise, CNVs of *RLG1a*, a tRNA ligase, have been associated with both flowering time and  
346 plant size in *M. guttatus* (Fig. 1b; Nelson *et al.*, 2019). Individual alleles have one to three, or, rarely,  
347 an extreme number (>250) of copies, and all alleles segregate following single locus expectations.  
348 Carriers of the 3-copy allele have delayed flowering and larger size relative to 1-copy allele  
349 homozygotes, while extreme-copy allele carriers flower earlier but are of similar size to the 1-copy  
350 allele homozygotes. Consistent with the findings for “large and slow” SNPs, the 3-copy allele carriers  
351 were most fit due to a female fecundity advantage in a year with a long spring. Conversely, the 1-  
352 copy homozygotes and the extreme allele carriers had highest survival and seed set in years with an  
353 early drought. Since larger plant size is likely a direct developmental consequence of delayed  
354 flowering, the “large and slow” alleles and the 3-copy CNV of *RLG1a* have (or tag causal variants  
355 that have) pleiotropic and antagonistic effects in different seasons, and these trade-offs maintain the  
356 polymorphisms.

357 Notably, these *M. guttatus* alleles also vary in frequency among populations (Troth *et al.*,  
358 2018), indicating that antagonistic pleiotropy may also contribute to local adaptation to spatially-  
359 varying selective pressures. Characterization of non-functional and functional *FRI* alleles in *A.*  
360 *thaliana* has revealed that this likely explains why functional *FRI* alleles are associated with drier  
361 habitats on average than non-functional *FRI* alleles. However, unlike the *M. guttatus* cases, the  
362 pleiotropy results from different downstream impacts of the polymorphism in different environments  
363 (Lovell *et al.*, 2013). Non-functional *FRI* alleles promote drought escape through rapid growth and  
364 early flowering. However, they also compromise water use efficiency because they cannot activate  
365 proline synthesis under drought stress through an *FLC*-dependent pathway, preventing drought  
366 tolerance.

367 These results lend evolutionary weight that reinforces the need to be mindful of evidence that  
368 mutants or natural variants in so-called flowering time genes often impact diverse other  
369 developmental and physiological traits (e.g., Pin & Nilsson, 2012; Ortiz-Marchena *et al.*, 2014; Auge  
370 *et al.*, 2019). Also, although pleiotropy is generally viewed negatively as a source of trade-offs or as a  
371 brake on the rate of adaptive evolution, it need not always be so. When an allele confers multiple  
372 favorable phenotypic effects, known as synergistic pleiotropy, then the overall selection coefficient  
373 will be larger, potentially facilitating divergence in the face of gene flow. For instance, recent work  
374 suggests that synergistic pleiotropy of alleles of *TWIN SISTER OF FT (TSF)*, which affect  
375 reproductive phenology but also impact branch number and the height:rosette diameter ratio in the  
376 field, can promote rapid divergence even at the microhabitat scale within populations of *A. thaliana*  
377 (Frachon *et al.*, 2017).

### 378 379 *The molecular basis of flowering time adaptation*

380 Another major question in this field is whether the adaptation process has predictable substitution  
381 biases (Stern & Orgogozo, 2009). Essentially, do certain mutation types preferentially contribute to  
382 adaptive evolution? One prominent hypothesis is that adaptation will be biased toward evolution  
383 through *cis*-regulatory changes since their effects can be restricted to particular tissues,  
384 developmental stages, or environments in contrast to coding sequence changes that may have  
385 pleiotropic impacts whenever a protein is expressed (Stern, 2000). Several meta-analyses supported  
386 this hypothesis, but others have argued that summarizing trends observed across traits and across  
387 all plants and animals, obscures important variation in the magnitude and heterogeneity of  
388 substitution biases (Streisfeld & Rausher, 2011). A large sample of causal natural variants affecting

389 flowering time and its environmental regulation provides a strong trait-specific dataset to address this  
390 problem. Most of these variants have been discovered in *A. thaliana* or other brassicas, but ever-  
391 improving resources in other plant groups should reduce this taxonomic bias going forward.

392 Coding, *cis*-regulatory, and CNVs all contribute to variation among natural populations, and  
393 all of these alleles eliminate or attenuate gene function, consistent with the bias that many more  
394 mutations will have loss-of-function rather than gain-of-function effects (Table 2). One notable trend  
395 is that coding variants, whether deletion or missense alleles, are associated with the losses of  
396 responses to environmental cues more often than *cis*-regulatory alleles. Many such mutations in *FLC*  
397 and *FRI* abolish vernalization response in the Brassicaceae. Likewise, a missense mutation  
398 segregating in *Brachypodium distachyon* compromises the function of an *FT* paralog (Woods *et al.*,  
399 2019). Expression of this paralog under short days is required to confer competency for floral  
400 induction when long days are experienced subsequently.

401 In contrast, *cis*-regulatory variants are more enriched among polymorphisms causing  
402 quantitative variation in flowering time or its environmental responsiveness. For instance, in both *A.*  
403 *thaliana* and *Capsella rubella*, a series of regulatory alleles of *FLC* are differentially sensitive to  
404 vernalization cues, with different alleles requiring different durations of cold exposure to be silenced  
405 and thus promote flowering (Coustham *et al.*, 2012; Li *et al.*, 2014, 2015; Yang *et al.*, 2018). In both  
406 *Arabidopsis arenosa* and *A. thaliana*, variants with more tandem repeats in the *CO* promoter flower  
407 later likely because they have an additional binding site for *CYCLING DOF FACTOR 1*, a repressor  
408 of *CO* expression (Fig. 1c; Rosas *et al.*, 2014; Baduel *et al.*, 2018). Convergent evolution involving  
409 the same repeat array may reflect a bias toward mutations that occur at higher rates, as much as a  
410 fixation bias for specific, limited allelic effects.

411 Together, these trends suggest that substitution biases in the type of mutations contributing  
412 to natural variation for this trait reflect the qualitative vs. quantitative nature of the favored trait  
413 variation more so than pleiotropy. Only examining SNP variation, a common GWAS approach, may  
414 often be insufficient. Insertion-deletion, CNVs, and even more complex rearrangements (e.g.,  
415 chimeric variation in tandem-duplicates of *MAF2*, Rosloski *et al.*, 2010) commonly cause phenotypic  
416 variation (Table 1; Fig. 1). Because these variant types arise by different mutational processes and  
417 impact local recombination rates, nearby SNPs may be ineffective in tagging them well by linkage  
418 disequilibrium (Schrider & Hahn, 2010).

419

#### 420 IV. Speciation

421 The evolution of flowering time is often highlighted as a potentially important contributor to the  
422 emergence of new plant species. As populations diverge in seasonal reproductive phenology, plants  
423 will more often be fertilized by pollen from their own population than by pollen from other populations,  
424 and this assortative mating constitutes a barrier to gene flow. Indeed, this process can confound  
425 GWAS and gene-environment association analysis, as variants causing flowering time divergence  
426 come to co-vary with population structure (e.g., Larsson *et al.*, 2013; Tyler *et al.*, 2016). Additional  
427 prezygotic and extrinsic postzygotic isolation results when flowering time divergence is driven by  
428 local adaptation or the timing of flowering of interpopulation hybrids reduces fitness relative to  
429 parental genotypes. Such processes fit with the mode of speciation known as ecological speciation,  
430 where reproductive isolation arises as a direct or indirect byproduct of divergent selection (Schluter,  
431 2009), and flowering time can be considered a so-called “magic trait” since its adaptive genetic  
432 divergence leads automatically to increased assortative mating (Taylor & Friesen, 2017).

433 Some of the earliest computer simulation models of speciation explored these possibilities,  
434 revealing how plastic differences in flowering time can facilitate speciation of adjacent, or parapatric,  
435 populations and how reinforcing selection can drive further flowering time divergence when  
436 interpopulation species hybrids form (Crosby, 1970; Stam, 1983; Dijk & Bijlsma, 1994). Consistent  
437 with these predictions, several empirical studies have found that flowering time differences contribute  
438 considerably to reproductive isolation between ecotypes or incipient species (e.g., Runquist *et al.*,  
439 2014; Sedeek *et al.*, 2014; Ferris *et al.*, 2017). Sympatric speciation theory shows that disjunct  
440 genetic clusters can emerge from a single, finite population on short evolutionary timescales even  
441 through non-selective processes when ample genetic variation for flowering time exists and  
442 individual plants flower for only brief periods over a long flowering season (Devaux & Lande, 2008).  
443 In addition, shifts in phenology may aid establishment of nascent polyploid species (Ramsey, 2011).  
444 Despite these conceptual advances and observations and also despite our detailed understanding of  
445 flowering time regulation, flowering time genes hardly contribute to the “speciation genes” literature  
446 (Rieseberg & Blackman, 2010). Nonetheless, several recent genetic studies have yielded results that  
447 bear upon predictions of verbal or analytical models of the speciation process.

448

#### 449 *Speciation with gene flow*

450 If flowering time differences do arise due to divergent selection and cause assortative mating that  
451 helps maintain phenotypic differentiation between species in sympatry, the causal flowering time loci  
452 should also remain differentiated even while divergence is eroded throughout most of the genome.



453 Recent work in the monkeyflower species pair of *M. guttatus* and *M. nasutus* has explored this  
454 possibility. These species are often found in sympatry and despite observed natural hybridization,  
455 they remain largely morphologically and developmentally distinguishable (Martin & Willis, 2007;  
456 Kenney & Sweigart, 2016). *M. guttatus* is an outcrosser and bee-pollinated, while *M. nasutus* is a  
457 predominant selfer.

458 Both species must experience a minimum critical photoperiod for floral induction, but *M.*  
459 *nasutus* can flower under shorter day lengths and thus earlier in the season than *M. guttatus*,  
460 (Fishman *et al.*, 2014). In the field, the temporal isolation caused at least in part by this change in  
461 photoperiodic regulation has been estimated to account for anywhere from ~4% to ~90% reduction in  
462 hybrid seed production relative to random mating, depending on the site and the direction of gene  
463 flow. Because the flowering period of  $F_1$  hybrids overlaps more with *M. guttatus*, introgression of *M.*  
464 *nasutus* alleles into *M. guttatus* is observed more often than the reverse (Martin & Willis, 2007).  
465 Genetic mapping reveals that two major effect QTL almost entirely explain the difference in critical  
466 photoperiod between the species (Fishman *et al.*, 2014). Consistent with a contribution to  
467 reproductive isolation in sympatry, the allelic diversity at one of these loci shows reduced  
468 introgression and is more differentiated between the species compared to the rest of the genome  
469 (Kenney & Sweigart, 2016).

470 Although this *Mimulus* example likely involved a fully allopatric phase at some point in the  
471 past, temporal isolation is of central importance in the best-known example of sympatric speciation in  
472 plants. Two palm species that evolved from a common ancestor on small, highly remote Lord Howe  
473 Island are primarily reproductively isolated by differences in soil type preference and flowering time.  
474 Temporal isolation through flowering displacement is 80% or 97% depending on the direction of gene  
475 flow (Hipperson *et al.*, 2016). Although some of this displacement is due to developmental plasticity  
476 that may have helped kickstart assortative mating by soil type (Gavrilets & Vose, 2007; Devaux &  
477 Lande, 2008), genetic differentiation also contributes to the species difference. Through differential  
478 expression analysis, selective sweep analysis, and functional tests of *A. thaliana* homologs,  
479 investigators have identified several candidate genes for this differentiation, including a homolog of  
480 the known flowering time regulator *FPA* (Dunning *et al.*, 2016). Because several of these genes are  
481 annotated with other functions that could respond to divergent selection on different soil types, it is  
482 tempting to speculate that synergistic pleiotropy may have facilitated both adaptive divergence and  
483 assortative mating, fitting the “magic trait” model under which sympatric speciation is most likely to

succeed (Servedio *et al.*, 2011). Expanded genomic resources for the *Howea* palms and functional studies in phylogenetically closer species may lend more credence to this possibility in the future.

#### Allopolyploidy and gene interactions

When a new polyploid individual evolves, it does so in sympatry with its progenitor(s) of lower ploidy. Consequently, to establish a new polyploid species, it must overcome being mated to extinction by its neighbors of different ploidy, a problem known as minority cytotype exclusion (Oswald & Nuismer, 2011). If temporal isolation is a direct consequence of the polyploidy event, then a new polyploid species is more likely to establish. Investigations into the control of flowering time in the allopolyploid *Arabidopsis suecica*, derived from *A. arenosa* and *A. thaliana*, demonstrate a mechanism by which such an immediate flowering displacement may emerge upon polyploidy (Wang *et al.*, 2006). The *A. thaliana* genome contributes a strong functional *FLC* allele and a non-functional *FRI* allele. Conversely, the *A. arenosa* genome contributes a weak *FLC* allele and a strong *FRI* allele. Hence, in the natural and newly synthesized allopolyploids, these two strong alleles interact epistatically such that *AaFRI* transactivates *AtFLC*, and the resulting high expression of *FLC* delays flowering relative to either diploid progenitor (Fig. 3).

#### IV. Evolution of developmental networks

Gene regulatory networks coordinate the complex orchestration of gene functions so that cells grow, divide, and adopt particular fates in a spatial-, temporal-, and environment-specific manner during development. By understanding why and how these networks become rewired or co-opted over time, we can learn how novel developmental programs evolve at both micro- and macroevolutionary scales. In addition, comparative studies of the networks regulating homologous traits in species that shared a common ancestor millions to hundreds of millions of years ago can reveal when gene functions are highly conserved; when circuit logic is conserved even if the specific proteins, RNAs, or *cis*-regulatory elements turnover, a process known as developmental system drift (Box 2); and how independent solutions to the same developmental problem can be reached entirely convergently. Finally, determining how network structures have evolved to confer robustness to genetic or environmental variability also has critical applications in conservation and agriculture.

By describing networks in multiple species to a level of mechanistic complexity that often yields important new insights into the fundamentals of gene regulation, flowering time research has repeatedly made seminal contributions to understanding these processes. For instance, early work

516 comparing the *GIGANTEA-CO-FT* regulatory circuit between *A. thaliana* and rice was among the first  
517 studies to demonstrate how the transcriptional relationships between homologous genes have been  
518 rewired to confer opposite behavior (*i.e.*, long-day vs. short-day response; Hayama *et al.*, 2003).  
519 Several recent papers have reviewed which aspects of flowering time regulation are conserved or  
520 differ among major study systems (Andrés & Coupland, 2012; Song *et al.*, 2015; Bouché *et al.*,  
521 2017). Therefore, we narrow our focus here to recent examples that illustrate particular conceptual  
522 advances achieved or supported through evolutionary developmental studies.

523

#### 524 *Versatility in gene networks facilitates life history evolution*

525 The perennial brassica *Arabidopsis alpina* has emerged as a powerful model system for examining how  
526 homologs of flowering time genes known from *A. thaliana* process information from internal and  
527 external timekeepers differently, resulting in its distinct life history (Fig. 4). *PERPETUAL*  
528 *FLOWERING1* (*PEP1*) is the *A. alpina* ortholog of *FLC*, and like *FLC*, it represses flowering unless  
529 the plant experiences sufficient vernalization. However, unlike *FLC*, which remains fully silenced  
530 post-vernalization, *PEP1* expression reverts to high levels in warm conditions and represses  
531 flowering in the meristems of young vegetative branches that will continue to grow and not flower  
532 until after the next winter. *PEP2*, orthologous to *APETALA 2* (*AP2*), is required to activate *PEP1* after  
533 vernalization (Lazaro *et al.*, 2019). The competency of *A. alpina* meristems to respond to  
534 vernalization cues is age-dependent; the expression of a small RNA, *miR156*, declines over  
535 developmental time and, in doing so, de-represses expression of *SQUAMOSA PROMOTER*  
536 *BINDING PROTEIN-LIKE 15* (*SPL15*), a promoter of flowering (Xu *et al.*, 2016; Hyun *et al.*, 2019).  
537 Age-dependent interactions between *PEP2* and another small RNA *miR172* also regulate axillary  
538 meristem competency independent of *SPL15* (Bergonzi *et al.*, 2013; Lazaro *et al.*, 2019)  
539 *SPL15* expression is also repressed by *PEP1* without vernalization and following reversion  
540 under warm conditions. Thus, *SPL15* integrates signals that confer competency so that axillary  
541 meristems that are too young and/or have not experienced cold do not produce flowers in *A. alpina*  
542 (Hyun *et al.*, 2019). In contrast, all meristems in *A. thaliana* are able to flower under warm, long days  
543 post-vernalization regardless of age because *FLC* is stably repressed, allowing *FT* induction to  
544 promote floral initiation. Congener and annual *Arabidopsis montbretiana* also flowers in post-vernalization,  
545 warm days by this mechanism because *AmFLC* is also stably repressed by vernalization. The same  
546 is true for a near isogenic line carrying *AmFLC* rather than *PEP1* in an otherwise *A. alpina*  
547 background and when *AmFLC* is transformed into *A. alpina pep1* mutants. Since *cis*-regulatory

548 variants can quantitatively alter the duration of cold necessary for stable *FLC* repression (Coustham  
549 *et al.*, 2012; Li *et al.*, 2014, 2015; Yang *et al.*, 2018), it is easy to conceive how natural selection  
550 could quickly modulate this capacity and shift the relative influence and redundancy of the age,  
551 vernalization, and photoperiod pathways for floral induction, driving evolutionary transitions in life  
552 history strategies while still preserving individual pathway structure.

#### 554 *Extensive turnover and convergence in developmental networks*

555 When we observe homologous phenotypes preserved across species separated by long evolutionary  
556 timescales, it is easy to assume that the regulatory interactions that structure the underlying  
557 developmental networks are also conserved. However, through either adaptive or neutral processes,  
558 developmental system drift frequently transpires (True & Haag, 2001). This process is the  
559 evolutionary equivalent of treading water. The underlying developmental mechanisms churn with  
560 change over time often without yielding a noticeable impact at higher scales of phenotypic  
561 organization due to the robustness of the system, leaving the overall impression of conservation.  
562 Indeed, ample empirical work in animal and fungal taxa has found, for instance, that the transcription  
563 factor binding sites can turnover rapidly even as gene expression levels remain conserved  
564 (e.g., Borneman *et al.*, 2007; Schmidt *et al.*, 2010; Berthelot *et al.*, 2018).

565 To our knowledge, only one comparative transcription-factor binding site study examining  
566 taxa of considerable evolutionary distance has been reported in plants. Mateos *et al.* (2017)  
567 compared the binding of *A. thaliana* *FLC* and its *A. alpina* ortholog *PEP1* to their respective  
568 organism's genomes through chromatin immunoprecipitation and high-throughput sequencing.  
569 Consistent with the extensive turnover observed in other taxa, only 28 of 204 bound genes (14%)  
570 shared direct target sequences in both species and only eleven more genes were commonly bound  
571 by both orthologs but at species-specific binding sites. Flowering time genes, as expected, were  
572 over-represented among the conserved targets. Interestingly though, both transcription factors  
573 directly bind and upregulate genes annotated as cold-responsive, but target almost entirely non-  
574 overlapping gene sets. Thus, these transcription factors serve at least two common functions,  
575 flowering and cold-response regulation, but for cold-response, there is a pattern potentially  
576 consistent with developmental system drift, assuming the *FLC* ortholog in the common ancestor of  
577 these two species also served this function. That is, an orthologous upstream regulator has  
578 maintained its functional role, yet has evolved to target a similar but not directly orthologous set of  
579 genes to produce a homologous trait.

580 Pathways may also misleadingly appear conserved between distant taxa due to convergent  
581 evolution. Homologous genes may gain a similar or the same role in a regulatory network multiple  
582 times independently, whether anew or reversing a previous loss along an evolutionary lineage. The  
583 regulation of *FT* homologs by *CO* homologs has emerged as one major case study of such high  
584 evolutionary lability (Ballerini & Kramer, 2011). Homologs of *CO* act as photoperiod-dependent  
585 regulators of *FT* homologs throughout the Brassicaceae and in rice, potato (*Solanum tuberosum*),  
586 sorghum, barley, strawberry (*Fragaria vesca*) and possibly soybean (Hayama *et al.*, 2003; Wu *et al.*,  
587 2014; Yang *et al.*, 2014; Mulki & Korff, 2016; Abelenda *et al.*, 2016; Kurokura *et al.*, 2017), though in  
588 some cases the regulation is partially or entirely indirect. However, substantial evidence in legumes  
589 (pea and *Medicago truncatula*), morning glory (*Ipomoea nil*), and poplar (*Populus* spp.) suggests that  
590 *CO* orthologs are not upstream regulators of *FT* in these species (Hayama *et al.*, 2007; Hsu *et al.*,  
591 2012; Wong *et al.*, 2014; Ridge *et al.*, 2016). Thus, *FT*-regulatory function appears to have been  
592 repeatedly evolutionary lost, gained, or both throughout the angiosperms. One gain of this function  
593 by *CO* likely occurred through coding and *cis*-regulatory changes following gene duplication near the  
594 origin of the Brassicaceae, as the sole *CO* homolog in the Cleomaceae, the sister clade to the  
595 Brassicaceae, functions similar to *COL1* and *COL2*, two *CO* paralogs that have circadian functions  
596 but do not impact photoperiodic flowering in *A. thaliana* (Simon *et al.*, 2015). Finally, in a particularly  
597 striking instance of convergent evolution, two independently transcribed genes, each of which  
598 encodes one of the two major protein functional domains found in *CO*, physically interact to regulate  
599 *FT* paralogs in sugar beet in a photoperiod-dependent manner (Dally *et al.*, 2018).

600 That different clades have independently evolved aspects of their photoperiod pathways is  
601 not surprising. Different taxonomic groups have adapted to colonize and thrive in temperate regions  
602 much more recently than they shared common ancestors. These independent histories of adaptation  
603 have also been invoked to explain the involvement of different phytochromes in photoperiod  
604 regulation and the complete lack of homology between the vernalization pathways of the temperate  
605 brassicas and grasses, for example (Chen *et al.*, 2014; Woods *et al.*, 2014). What is surprising is that  
606 *CO* homologs are repeatedly recruited or disconnected somehow. One explanation for this pattern  
607 may be that *CO* homologs have a strongly conserved function in photoperiodic regulation of another  
608 fundamental function like carbohydrate metabolism (Serrano *et al.*, 2009; Ortiz-Marchena *et al.*,  
609 2014), and thus they are predisposed to be co-opted for novel photoperiodic adaptations. Because  
610 *CO* promotes transcription both by directly binding DNA and as a co-activator (Blackman & Michaels,  
611 2010), *CO* homologs may also have more routes to gain new functions compared to other gene

612 families. Lastly, because photoperiodic flowering often involves multiple mechanisms acting together  
613 to repress flowering in non-inductive photoperiods and promote flowering in inductive photoperiods,  
614 and since several other pathways converge at *FT* transcriptional regulation, the *CO-FT* regulatory  
615 relationship may be especially prone to loss through developmental system drift when rendered  
616 redundant or irrelevant by shifts in control elsewhere in the network.

#### 618 *Network elaboration through gene duplication*

619 Gene duplication is a prominent driver of developmental system drift and convergence, as different  
620 members of gene families with similar functional capacities may swap in and out of pathways in  
621 diverging taxa over time. In addition, gene family expansion has long been postulated to be an  
622 important, potent source of evolutionary novelty in gene networks. On arrival, new gene duplicates  
623 are often partially or wholly functionally redundant to the ancestral gene copy. They may bind to the  
624 same *cis*-regulatory sequences, interact with the same protein complexes, or process the same  
625 metabolic compounds. Consequently, until the redundancy of young duplicate pairs is fully resolved  
626 through loss of one copy or subfunctionalization, new duplicates are well positioned to evolve new  
627 cooperative or competitive interactions with the ancestral gene copy or other paralogs that elaborate  
628 gene networks.

629 To our knowledge, studies of recent lineage-specific duplicates of *FT* were the first to  
630 demonstrate the mettle of this theory empirically. In particular, young *FT-like* genes in several  
631 species have evolved anti-florigenic regulatory activities, likely by competing with *FT* for partners in  
632 the florigen activating complex in the shoot apical meristem. This mechanism was first suggested by  
633 findings in sunflower (Blackman *et al.*, 2010). A frameshift allele of a recently duplicated, meristem-  
634 expressed *FT* copy rose to high frequency during sunflower domestication, and this allele acts in a  
635 dominant negative manner by interfering with the capacity of a different *FT* paralog to promote  
636 flowering in an *A. thaliana ft* mutant background. In tobacco, several recently duplicated *FT* paralogs  
637 that act to delay flowering have mobile transcripts and are capable of binding the tobacco *FD*  
638 paralog, lending support to the hypothesis that they inhibit *FT* function through competition for  
639 activation complex partners (Harig *et al.*, 2012; Huang *et al.*, 2018a). An *FT* paralog in soybean  
640 (*GmFT1a*) may similarly antagonize the action of florigenic *FT* paralogs (Liu *et al.*, 2018). Additional  
641 evidence that developmental networks may be adaptively elaborated through novel antagonistic  
642 protein interactions between young duplicates and ancestral paralogous copies has now emerged in  
643 other systems (e.g., Charrier *et al.*, 2012; Dennis *et al.*, 2012).

644 In several other cases, lineage-specific *FT* duplicates have evolved to antagonize the  
645 canonical *FT* paralog's function not by competing for protein interactions but instead by repressing its  
646 transcription. This mode of evolved antagonism was first discovered in sugar beet (Pin *et al.*, 2010),  
647 and subsequent studies of *FT* paralogs that suppress photoperiod-induced responses in onion  
648 (*Allium cepa* L.) and tomato have described similar mechanisms of action (Lee *et al.*, 2013; Soyk *et*  
649 *al.*, 2017). The evolution of lineage-specific *FT* homologs also illustrates how gene duplication can  
650 facilitate the evolution of novelty through co-option. *FT* paralogs distinct from those that function in  
651 the environmental regulation of flowering have evolved to act as photoperiod-specific regulators of  
652 bulb and tuber formation in onion and potato, respectively (Navarro *et al.*, 2011; Lee *et al.*, 2013).

653

## 654 **V. Conclusions and future directions**

655 Our goal has been to highlight how investigating the genetics of flowering time diversity has enriched  
656 our understanding of fundamental evolutionary processes. Several discoveries summarized above  
657 reveal how diverse types of variants have repeatedly altered the pathways that regulate the  
658 developmental plasticity of flowering time as the seasonal phenologies of populations or crops have  
659 adapted to local climate variation. Many examples of convergent genetic evolution are noted,  
660 indicating that “evolutionary hotspots” often harbor adaptive genetic variation in this trait. However,  
661 additional studies in *Arabidopsis*, maize and *Mimulus* suggest that polygenic adaptation may also be  
662 common, particularly if standing variation within populations is characterized by antagonistic  
663 pleiotropy across years or locations. Recent case studies also illustrate how flowering time facilitates  
664 ecological speciation with gene flow and polyploid speciation. Finally, comparisons of regulatory  
665 pathways among distant taxa have found extensive rewiring through turnover and convergence at  
666 several hierarchical scales and demonstrated how gene duplications foster developmental network  
667 innovation through multiple mechanisms.

668 Future efforts to identify the molecular basis of flowering time diversity will undoubtedly  
669 continue to advance the field of evolutionary biology. Current technologies for rapidly and cheaply  
670 generating heaps of population and functional genomic data are now often readily transferable  
671 across systems or applicable to archaeological and herbarium samples. Genome editing tools hold  
672 great promise for confirming gene and allele functions in a broader range of taxa. A point  
673 underscored by several studies reviewed above, however, is that there remains no substitute for  
674 multi-year, multi-site field studies for understanding how alleles function to produce trait variation and  
675 interact with selection in native environments. Bridging all these approaches is increasingly important

676 if we hope to predict how quickly and fully the flowering time diversity segregating within species will  
677 foster adaptation to non-analog combinations of seasonal cues and selective agents in future  
678 climates.

679 As new mechanisms of flowering time plasticity to drought, nutrient stress, ambient CO<sub>2</sub>, and  
680 soil microbiota become better described, genetic investigations of their diversity should reveal how  
681 pleiotropic or modular adaptive variation affecting these responses can be (Blackman, 2017).  
682 Comparative study of these pathways may be particularly critical for learning how unique life histories  
683 like gregarious flowering or masting evolve (Peng *et al.*, 2013; Kobayashi *et al.*, 2013). Another area  
684 where flowering time is particularly poised to contribute is evolutionary epigenetics. Epi-allelic  
685 variation in flowering can be generated artificially (Cortijo *et al.*, 2014) but flowering epi-alleles  
686 adaptive in the wild remain undescribed. Given past theoretical findings, it is also tempting to  
687 speculate how epi-alleles for flowering could kickstart speciation, a possibility with precedence in  
688 postzygotic incompatibilities (Blevins *et al.*, 2017). Furthermore, comparisons of flowering time  
689 evolution in selfing and outcrossing species will illuminate how mating system influences the genetic  
690 architecture of adaptation and the reliance of adaptation on *de novo* mutations vs. standing variation  
691 (Glémin & Ronfort, 2013). Finally, more phylogenetically structured sets of network analyses are  
692 needed to determine which network properties promote developmental system drift and whether the  
693 exploration of genotypic space that occurs by this process fosters the origin of novel functions.

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1121 1642.
- 1122

1124 **Box 1: Connecting Genotype to Phenotype**

1125 Most studies reviewed here identified allelic variation affecting flowering time starting with either  
1126 quantitative trait locus (QTL) mapping or a genome wide association study (GWAS). QTL mapping  
1127 tests for linkage between genetic markers and trait variation in controlled crosses derived from a  
1128 limited number of parents. QTL detection power and mapping precision depend on marker density,  
1129 population size, and the number of generations of recombination since the initial cross. In many  
1130 species, QTL mapping has been limited to one or two biparental crosses, sampling too few  
1131 genotypes to yield a population- or species-wide picture of a trait's genetic architecture. Moreover,  
1132 the challenging work to go from broad genomic intervals to causal variants is never completed for  
1133 most QTLs.

1134 GWAS overcomes some of these limitations. Hundreds of genotypes are sequenced,  
1135 sampling much more diversity and many generations of recombination that have eroded trait-marker  
1136 associations except at the causal polymorphism and the most tightly linked variants. Power to detect  
1137 marker effects depends on the sample size of each genotypic class, and consequently, GWAS is  
1138 most powerful for detecting common, large-effect alleles. Population structure and kinship must be  
1139 controlled for to reduce false positives. Even with whole genome re-sequencing data, coverage is  
1140 often too low to include copy-number or presence-absence variants, and many studies ignore  
1141 haplotype and insertion-deletion information, complicating candidate gene and causal variant  
1142 identification.

1143 Neither of these approaches reveals whether allelic variation was shaped by past adaptive  
1144 processes. Population genomic analyses are needed to infer selective sweeps or test for  
1145 associations between allele frequency and environmental variation. Conversely, while population  
1146 genomics studies on their own may highlight adaptive variation at homologs of flowering regulators  
1147 (e.g., Keller *et al.*, 2012; Pyhäjärvi *et al.*, 2013), additional work remains necessary to connect  
1148 sequence and flowering time variation, as these genes often regulate additional traits.

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1155 **Box 2 - Glossary**

1156 **Artificial selection:** Human-imposed selection for desired phenotypes and the maintenance of those  
1157 phenotypes in the population.

1158

1159 **Convergent evolution:** Repeated origin of similar phenotypes in independent evolutionary lineages.

1160

1161 **Balanced polymorphism:** Multiple alleles at a single locus are actively maintained by selection over  
1162 many generations within a population.

1163

1164 **Antagonistic pleiotropy:** Allelic variation at a single locus where different alleles have the highest  
1165 relative fitness in different environments.

1166

1167 **Synergistic pleiotropy:** Allelic variation at a single locus that confers beneficial effects through  
1168 multiple phenotypes.

1169

1170 **Ecological speciation:** Reproductive isolation evolves as a by-product of divergent selection acting  
1171 on populations adapting to different environments

1172

1173 **Developmental system drift:** Divergence through time of gene regulatory networks governing the  
1174 development of a homologous trait even as the phenotype itself remains conserved

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## 1179 **Figure Legends**

1180

### 1181 **Figure 1**

1182 Case studies at three scales illustrate how change in DNA copy number is associated with variation  
1183 in phenology. (a) In maize, decreasing genome size and heterochromatin knob count with elevation  
1184 is correlated with earlier flowering. Larger genome sizes are associated with slower cell production  
1185 rates and thus likely slower developmental progress toward flowering. Data adapted from Bilinski *et*  
1186 *al.* 2018. (b) In *M. guttatus*, copy number variation in a tRNA ligase is associated with variation in  
1187 flowering time, and the relative fitness of these alleles differs across years. Data adapted from  
1188 Nelson *et al.* 2019. (c) In *A. thaliana*, copy number variation in a cis-regulatory element in the CO  
1189 promoter causes changes in flowering time and interacts with the presence/absence of a functional  
1190 FRI allele. Data adapted from Rosas *et al.* 2014.

1191

### 1192 **Figure 2**

1193 Polygenic adaptation during maize domestication and dispersion. (a) Regulatory relationships of  
1194 genes involved in the evolution of photoperiodic flowering during maize domestication and  
1195 dispersion. (b) A cis-regulatory allele of *ZCN8* (dark blue) became common as maize was  
1196 domesticated and initially dispersed beyond the Balsas River basin (yellow star). Next, an additional  
1197 variant in *ZCN8* and variants affecting *Vgt1*, *ZmCCT9*, *ZmCCT10* and *ZmMADS69* (light blue)  
1198 allowed for further northward expansion of maize cultivation in North America.

1199

### 1200 **Figure 3**

1201 Epistatic interactions between sub-genomes affect flowering following polyploidy. In allopolyploid  
1202 *Arabidopsis suecica*, a functional vernalization pathway is restored through interactions between  
1203 gene copies from both the *A. arenosa* and *A. thaliana* parental genomes. The strong *AaFRI* allele  
1204 transactivates the strong *AtFLC* alleles to repress flowering unless plants experience sufficient  
1205 vernalization.

1206

### 1207 **Figure 4**

1208 Rewiring of flowering time regulatory pathways accounts for differences between annual and  
1209 perennial life histories. (a) In the perennial *Arabis alpina*, cold exposure (blue interactions) and aging  
1210 (green interactions) are both required to promote floral induction. (b) In *Arabidopsis thaliana*, long

1211 days (orange interactions) can promote flowering independently of cold exposure (blue interactions)  
1212 and aging (green interactions).

**Table 1. Flowering Time Genes Contributing to Crop Domestication and Dispersion**

Phenotypic effect	Species	Common Name	Gene	Variant(s)	Reference(s)
Reduced photoperiod and vernalization response in long-day crops	<i>Hordeum vulgare</i>	Barley	<i>VRN2-H2</i>	deletion	(Yan <i>et al.</i> , 2004)
	<i>Hordeum vulgare</i>	Barley	<i>Ppd-H1</i>	coding sequence mutation	(Turner <i>et al.</i> , 2005)
	<i>Triticum monococcum</i>	Einkorn Wheat	<i>VRN2</i>	deletion; coding sequence mutation	(Yan <i>et al.</i> , 2004)
	<i>Triticum monococcum</i>	Einkorn Wheat	<i>VRN1</i>	5' cis-regulatory variation	(Yan <i>et al.</i> , 2003, 2004)
	<i>Triticum aestivum ssp. aestivum</i>	Winter Wheat	<i>Ppd-H1</i>	copy number variation; homeolog combinations	(Bentley <i>et al.</i> , 2013; Würschum <i>et al.</i> , 2015, 2018)
	<i>Pisum sativum</i>	Pea	<i>ELF3</i>	coding sequence mutation	(Weller <i>et al.</i> , 2012)
	<i>Lens culinaris</i>	Lentil	<i>ELF3</i>	coding sequence mutation	(Weller <i>et al.</i> , 2012)
Changes in photoperiod requirements in short-day crops	<i>Solanum lycopersicum</i>	Tomato	<i>SP5G</i>	3' UTR cis-regulatory variation	(Soyk <i>et al.</i> , 2017; Zhang <i>et al.</i> , 2018)
	<i>Sorghum bicolor</i>	Sorghum	<i>FT</i>	5' cis-regulatory variation	(Cuevas <i>et al.</i> , 2016)
	<i>Glycine max</i>	Soybean	<i>FT2c</i>	TE insertion in intron	(Wu <i>et al.</i> , 2017)
	<i>Helianthus annuus</i>	Sunflower	<i>HaFT1</i>	coding sequence mutation	(Blackman <i>et al.</i> , 2010)
	<i>Zea mays L.</i>	Maize	<i>ZCN8</i>	5' cis-regulatory variation	(Guo <i>et al.</i> , 2018)
	<i>Zea mays L.</i>	Maize	<i>Vgt1</i>	TE insertion in coding region, coding sequence mutation	(Ducrocq <i>et al.</i> , 2008)
	<i>Zea mays L.</i>	Maize	<i>ZmMADS69</i>	unknown	(Liang <i>et al.</i> , 2019)
	<i>Zea mays L.</i>	Maize	<i>ZmCCT9</i>	TE insertion in 5' regulatory region	(Huang <i>et al.</i> , 2018b)
	<i>Zea mays L.</i>	Maize	<i>ZmCCT10</i>	TE insertion in 5' regulatory region	(Hung <i>et al.</i> , 2012; Yang <i>et al.</i> , 2013)
	<i>Zea mays L.</i>	Maize	<i>ZmCCT10</i>	TE insertion in 5' regulatory region	(Hung <i>et al.</i> , 2012; Yang <i>et al.</i> , 2013)

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<i>Oryza sativa</i>	Rice	<i>PRR37</i>	coding sequence mutation	(Koo <i>et al.</i> , 2013)
<i>Oryza sativa</i>	Rice	<i>Ghd7</i>	deletion; coding sequence mutation	(Xue <i>et al.</i> , 2008; Yan <i>et al.</i> , 2013)
<i>Glycine max</i>	Soybean	<i>PHYA</i>	deletion; coding sequence mutation	(Xu <i>et al.</i> , 2013; Jiang <i>et al.</i> , 2014)
<i>Phaseolus vulgaris</i>	Common bean	<i>PHYA3</i>	coding sequence mutation	(Weller <i>et al.</i> , 2019)

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**Table 2. Flowering Time Genes Contributing to Natural Variation among Wild Populations<sup>1</sup>**

Phenotypic Effect	Species	Gene	Substitution Type <sup>2</sup>	Reference(s)
Loss of Vernalization Response	<i>Arabidopsis thaliana</i>	<i>FRI</i>	Coding (20)	(Johanson <i>et al.</i> , 2000; Shindo <i>et al.</i> , 2005; Strange <i>et al.</i> , 2011)
	<i>Arabis alpina</i>	<i>PEP1</i>	Coding (4), Regulatory (1)	(Albani <i>et al.</i> , 2012)
	<i>Capsella rubella</i>	<i>FLC</i>	Regulatory (2), Coding (1) <sup>3</sup>	(Guo <i>et al.</i> , 2012; Yang <i>et al.</i> , 2018)
	<i>Boechera stricta</i>	<i>FLC</i>	Coding (1)	(Lee <i>et al.</i> , 2018)
	<i>Arabidopsis arenosa</i>	<i>FLC</i>	Coding (2)	(Badel <i>et al.</i> , 2018)
Varying Duration of Cold Required for Vernalization	<i>Arabidopsis thaliana</i>	<i>FLC</i>	Regulatory (5)	(Coustham <i>et al.</i> , 2012; Li <i>et al.</i> , 2014, 2015)
Varying Sensitivity to Ambient Temperature	<i>Arabidopsis thaliana</i>	<i>FLM</i>	Regulatory (8)	(Lutz <i>et al.</i> , 2015, 2017)
Loss of Short-Day Vernalization Response	<i>Brachypodium distachyon</i>	<i>FTL9</i>	Coding (2)	(Woods <i>et al.</i> , 2019)
Early Flowering in Short Days	<i>Arabidopsis thaliana</i>	<i>FT</i>	Regulatory (2)	(Bao <i>et al.</i> , 2019)
	<i>Arabidopsis thaliana</i>	<i>MAF2</i>	Coding (1)	(Rosloski <i>et al.</i> , 2010)
	<i>Arabidopsis thaliana</i>	<i>SVP</i>	Coding (1)	(Méndez-Vigo <i>et al.</i> , 2013)
	<i>Arabidopsis thaliana</i>	<i>PHYC</i>	Coding (2) <sup>3</sup>	(Balasubramanian <i>et al.</i> , 2006)
	<i>Arabidopsis thaliana</i>	<i>CO</i>	Regulatory (3) <sup>3</sup>	(Rosas <i>et al.</i> , 2014)
Early Flowering in Long Days	<i>Arabidopsis arenosa</i>	<i>CO</i>	Regulatory (2)	(Badel <i>et al.</i> , 2018)
	<i>Arabidopsis thaliana</i>	<i>FLC</i>	Regulatory (7)	(Lempe <i>et al.</i> , 2005; Quadrana <i>et al.</i> , 2016)
	<i>Arabidopsis thaliana</i>	<i>FRL1</i>	Coding (1) <sup>3</sup>	(Schläppi, 2006)



Early Flowering in Constant Light	<i>Arabidopsis thaliana</i>	<i>FRL2</i>	Coding (1) <sup>3</sup>	(Schläppi, 2006)
	<i>Arabidopsis thaliana</i>	<i>PHYD</i>	Coding (1) <sup>3</sup>	(Aukerman <i>et al.</i> , 1997)

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<sup>1</sup>Only genes where flowering time function has been experimentally verified are listed. <sup>2</sup>Number of independent allelic variants discovered by type is given in parentheses. <sup>3</sup>One or more of the allelic variants has only been observed in one accession.

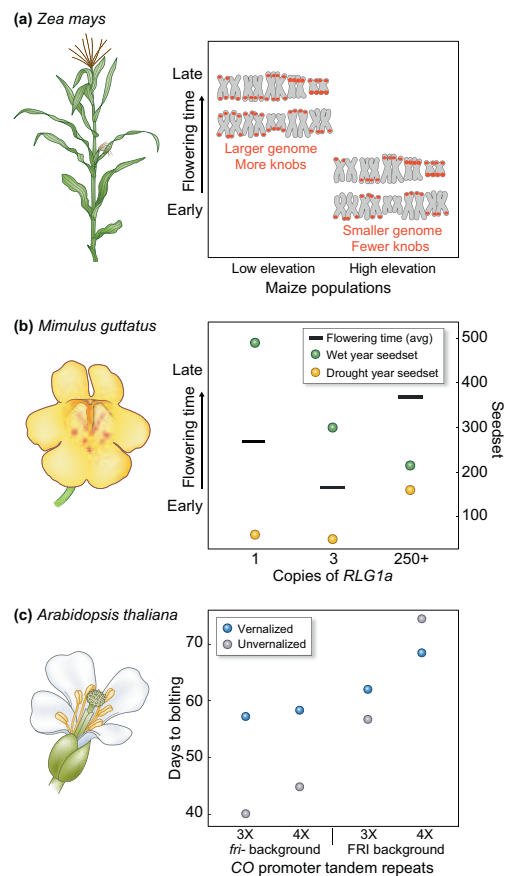


Figure 1

Tansley Review 30442

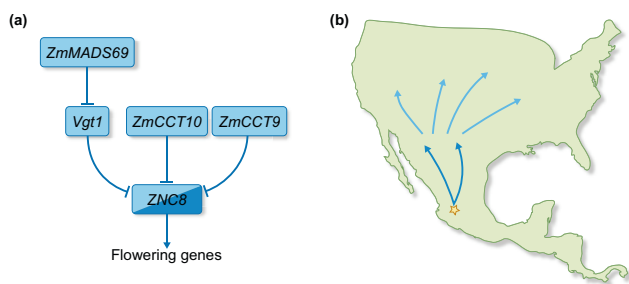


Figure 2

Tansley Review 30442

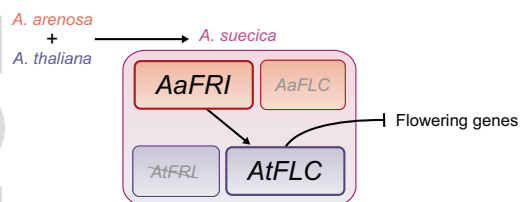


Figure 3

Tansley Review 30442

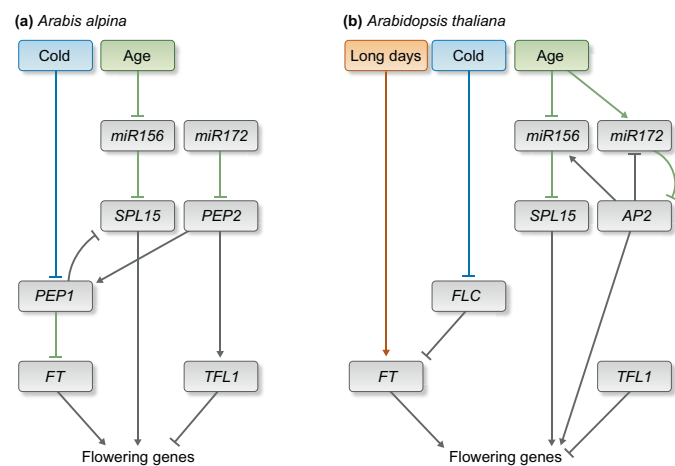


Figure 4

Tansley Review 30442